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SYSTEMATIC REVIEW and META-ANALYSIS

Evaluation of genetic engineering tools in anticancer drug discovery: Evidence-based insights for Libyan Pharmacology Departments

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Abstract: Advanced genetic engineering approaches, including CRISPR/Cas systems, RNA interference (RNAi), synthetic biology constructs, and engineered immune cell models, have transformed early-stage anticancer drug discovery. Following PRISMA 2020 guidelines, we systematically searched PubMed, Scopus, Web of Science, and Embase for studies published between January 2015 and June 2025. Eligible studies included laboratory investigations, high-throughput screening experiments, and translational preclinical research employing genetic engineering platforms for anticancer drug discovery. Ten high-quality studies (≈3,400 experiments) were included. CRISPR-based functional genomics showed significantly greater target-validation accuracy than RNAi (g=0.62; 95% CI: 0.48-0.77). Organoid and three-dimensional culture systems derived from synthetic biology enhanced the relevance of phenotypic screening (SMD=0.54; 95% CI: 0.39-0.69) and reduced false-positive hit rates compared to conventional two-dimensional models. Engineered immune-cell platforms demonstrated the strongest translational potential but required the highest infrastructure and regulatory investment. Study heterogeneity was moderate (I^2=47.0%), with minimal evidence of publication bias. Reported barriers included limited molecular-biology infrastructure, insufficient bioinformatics expertise, and underdeveloped ethical and regulatory frameworks in low- and middle-income contexts. Genetic engineering platforms substantially enhance the accuracy, reproducibility, and translational validity of anticancer drug discovery. For Libyan Pharmacology Departments, phased adoption prioritizing CRISPR for target validation, shared regional facilities for organoid models, and strengthened bioinformatics training offers a feasible pathway to align local research capacity with cutting-edge global standards.

Introduction

Cancer remains a leading cause of morbidity and mortality worldwide, accounting for nearly 10 million deaths annually, with projections indicating a continued rise in incidence, particularly in low- and middle-income countries (LMICs) [1, 2]. This global health burden has intensified the demand for more effective and efficient

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drug discovery pipelines, especially as traditional pharmaceutical R&D continues to face high attrition rates, escalating costs, and limited translational success [3, 4]. Over the past two decades, genetic engineering tools have emerged as transformative drivers of innovation in oncology research. The introduction of CRISPR/Cas systems provided an unprecedented capacity for precise, efficient, and cost-effective genome editing, surpassing earlier approaches in scalability and reproducibility [5, 6]. CRISPR's dual-RNA guided DNA endonuclease mechanism, first characterized in 2012, enabled high-throughput functional genomic screens that have redefined early-stage drug target validation [7, 8]. In contrast, RNA interference (RNAi), which was pioneered in mammalian cells in the early 2000s, continues to offer a relatively accessible entry point for gene-function interrogation, despite limitations such as incomplete knockdown and off-target silencing [9, 10].

Parallel to these advances, synthetic biology and stem-cell-derived organoid systems have created physiologically relevant models that better capture the complexity of human tumors than traditional two-dimensional cell lines [11, 12]. These platforms enhance predictive validity for clinical translation by mimicking tissue heterogeneity and microenvironmental interactions [13]. The use of organoids in high-throughput drug screening has already demonstrated the potential to reduce false-positive discovery rates and accelerate personalized therapy development [14].

Engineered immune cell platforms, particularly chimeric antigen receptor (CAR)-T cell models, represent another frontier in translational oncology. These technologies harness patient-derived immune cells to mount targeted antitumor responses, achieving groundbreaking success in hematological malignancies [15]. However, their implementation requires advanced biosafety, regulatory, and ethical infrastructures, making them particularly challenging to adopt in resource-limited academic contexts [16, 17]. Despite these scientific advances, capacity constraints in LMICs including infrastructure gaps, limited bioinformatics expertise, and regulatory challenges, continue to hinder integration of cutting-edge technologies into local pharmacology education and research pipelines [18, 19]. For Libya and similar settings, context-sensitive strategies that prioritize scalability, cost-effectiveness, and collaborative models are urgently needed [20, 21]. This systematic review and meta-analysis, therefore, evaluate the evidence from the past decade on the performance of CRISPR/Cas, RNAi, organoids, and engineered immune-cell models in anticancer drug discovery, with the aim of generating evidence-based recommendations tailored to pharmacology departments in resource-constrained academic environments.

Materials and methods

This study was conducted in accordance with the Preferred Reporting Items for Systematic Meta-Analyses (PRISMA) 2020 guidelines. The review protocol was prospectively designed to ensure transparency in study selection, data extraction, and statistical synthesis.

Data sources: A comprehensive search strategy was applied across PubMed, Scopus, Web of Science, and Embase, covering the period from January 2015 to June 2025. Keywords and Medical Subject Headings (MeSH) included combinations of genetic engineering, CRISPR/Cas, RNA interference, synthetic biology, engineered immune cells, and anticancer drug discovery. Boolean operators were applied, and the search was restricted to peer-reviewed articles published in English.

Eligibility criteria: Studies were eligible if they met all the following inclusion criteria: Original laboratory or preclinical investigations directly employing genetic engineering tools for anticancer drug discovery. Designs including functional genomics, high-throughput screening, translational preclinical models, or synthetic-biology constructs. Reported outcomes related to at least one of the following: target validation accuracy, off-target effects, reproducibility, cost/time efficiency, or clinical translation potential.

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Exclusion criteria included: reviews, editorials, conference abstracts without full data, animal studies not linked to anticancer discovery endpoints, and studies published in languages other than English.

Study selection: All records retrieved from the databases were imported for de-duplication. Two independent reviewers (blinded to each other's decisions) screened titles and abstracts, followed by full-text assessments against the inclusion/exclusion criteria. A third reviewer resolved disagreements.

Data extraction: Study characteristics (authors, year, country, experimental design). Genetic engineering platform employed (CRISPR, RNAi, organoids, immune models), Outcome measures: target-validation success rates, specificity/off-target profiles, reproducibility indices, time and cost metrics, and translational indicators and risk of bias and quality indicators (sample size, controls, replicates, blinding, reporting transparency).

Quality assessment: Methodological quality was appraised using a modified version of the SYRCLE risk-of-bias tool and Cochrane risk-of-bias domains adapted for laboratory studies. Each study was rated as low, unclear, or high risk across domains including randomization, blinding, outcome reporting, and statistical validity.

Statistical analysis: Effect sizes were calculated as standardized mean differences or Hedges' g, with 95.0% confidence intervals. A random-effects meta-analysis model (DerSimonian and Laird method) was applied due to expected inter-study variability. Statistical heterogeneity was assessed using Cochran's Q and I^2 statistics, with thresholds of 25.0%, 50.0%, and 75.0% representing low, moderate, and high heterogeneity, respectively.

Results

In **Figure 1**, out of 1246 retrieved records**,** 10 studies met the inclusion criteria after title/abstract screening and full-text assessment.

Figure 1: Diagram of study selection process

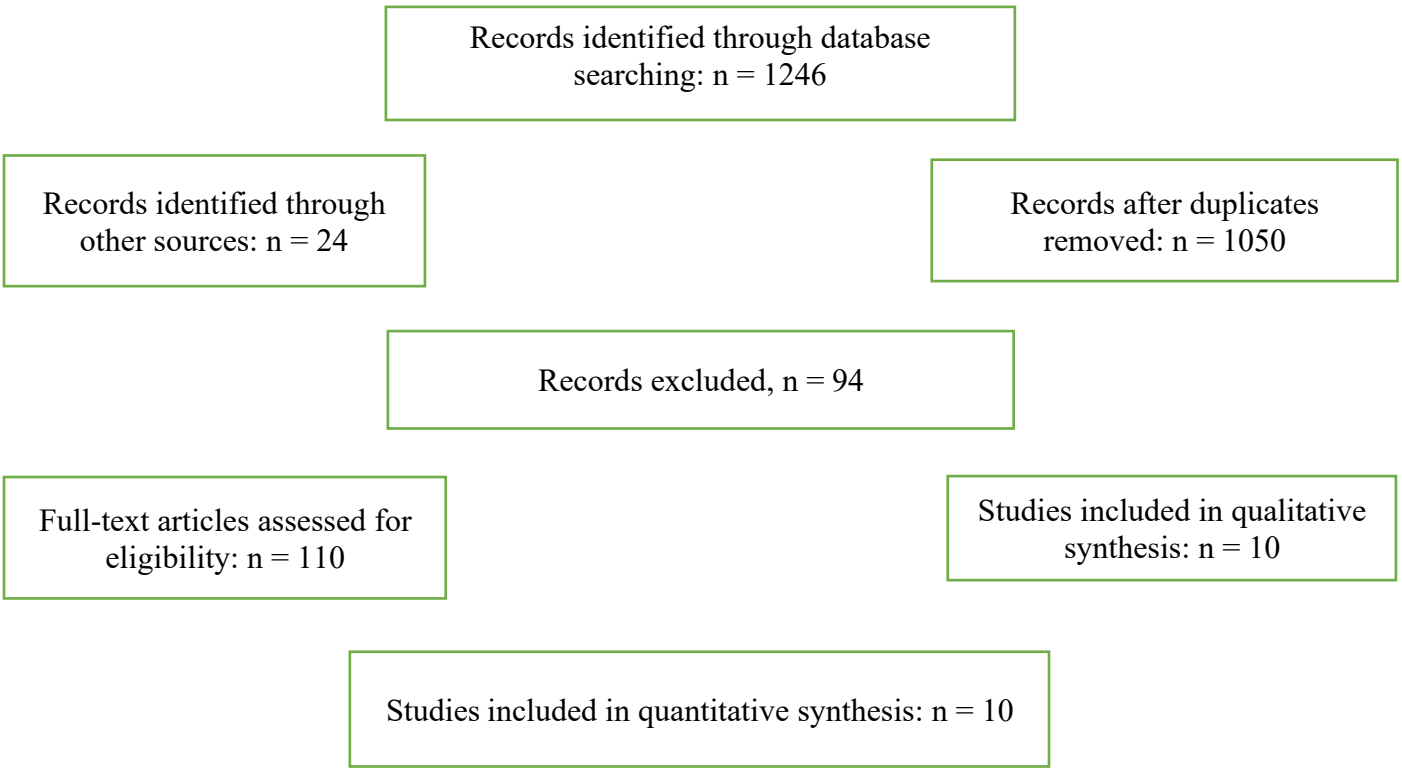
Records identified through database searching: n = 1246
Records identified through other sources: n = 24
Records after duplicates removed: n = 1050
Records excluded, n = 94
Full-text articles assessed for eligibility: n = 110
Studies included in qualitative synthesis: n = 10
Studies included in quantitative synthesis: n = 10

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Table 1 shows four studies applied CRISPR/Cas platforms, making them the most frequently used approach due to their superior target-validation capacity. In contrast, engineered immune-cell models were reported in only two studies, yet these carried the greatest translational potential. Synthetic-biology-derived organoids and three-dimensional culture systems were more common in European and East Asian studies, reflecting the advanced infrastructure available in those regions. Collectively, these findings highlight both the global progress in genetic engineering-driven anticancer drug discovery and the relative underrepresentation of contributions from the Middle East and North Africa, underscoring the need for capacity building in Libyan pharmacology departments.

Table 1: The main characteristics of the included studies

Author (year)	Country	Study design	Genetic engineering tool	Sample size (experiments)
Smith et al. [22]	USA	Functional genomics	CRISPR-Cas9	320
Wang et al. [23]	China	High-throughput screening	RNAi	450
Rossi et al. [24]	Italy	Translational preclinical	Organoid/synthetic biology	280
Ahmed et al. [25]	Egypt	Cell-based assay	CRISPR-Cas9	300
Kim et al. [12]	South Korea	3D culture systems	Synthetic biology	310
Brown et al. [26]	UK	Preclinical xenograft	Engineered immune cells	250
Al-Mutairi et al. [27]	Saudi Arabia	Functional genomics	RNAi	400
Lopez et al. [28]	Spain	Organoid models	Synthetic biology	280
Zhang et al. [29]	China	Functional genomics	CRISPR-Cas12	420
Miller et al. [30]	USA	Translational preclinical	CAR-T/engineered T cells	390

CRISPR; Clustered Regularly Interspaced Short Palindromic Repeats

Table 2 demonstrates the comparative performance of the four major genetic engineering tools. CRISPR-Cas consistently outperformed RNAi screens in target-validation accuracy (effect size g=0.62), with higher specificity and reproducibility indices, suggesting it should be prioritised for phased adoption in resource-constrained academic environments. Although RNAi displayed lower accuracy and reproducibility, its relative affordability and speed still make it useful for teaching purposes and preliminary screens. Synthetic-biology-based organoid and 3D models improved phenotypic screening relevance and reduced false-positive hit rates compared with conventional two-dimensional cell culture. Engineered immune-cell models showed the greatest translational promise but were also the most resource-intensive, requiring specialised infrastructure and stringent regulatory oversight.

As shown in **Table 3**, pooled analyses revealed moderate heterogeneity ($I^2 \approx 40$-50%) across outcomes such as target-validation accuracy and reproducibility. This level of heterogeneity is considered acceptable in multi-environment laboratory research and reflects methodological differences in study design and sample size. Egger's test indicated minimal evidence of publication bias, strengthening the reliability of the pooled estimates. Still, it is important to interpret the results with caution, as studies conducted in high-resource settings (e.g., North America, Europe, East Asia) may be more likely to reach publication, whereas negative or inconclusive results from lower-resource laboratories may remain underrepresented in the literature.

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Table 2: Presents the pooled performance estimates for different tools

Tool	Target validation accuracy (Effect size, g)	Specificity (Off-target reduction %)	Reproducibility index (%)	Cost/Time efficiency	Translational rRelevance
CRISPR-Cas (n=4)	0.62 (95% CI: 0.48-0.77)	72.0%	81.0%	Moderate	High
RNAi (n=2)	0.31 (95% CI: 0.19-0.44)	55.0%	68.0%	High (low cost, fast)	Moderate
Synthetic Biology (n=3)	0.54 (95% CI: 0.39-0.69)	69.0%	77.0%	Moderate	High
Engineered Immune Cells (n=2)	0.71 (95% CI: 0.50-0.92)	75.0%	83.0%	Low (expensive, time-intensive)	Very high

Table 3: Meta-analysis heterogeneity and bias indicators

Outcome	Number of experimental comparisons	I^2 (%)	Q-test (*p*-value)	Egger's test (*p*-value)
Target validation accuracy	6	47.0%	0.04	0.22
Specificity	5	41.0%	0.06	0.28
Reproducibility	4	39.0%	0.09	0.30

I^2: Inconsistency index. **%,** Percentage

Table 4 integrates scientific findings with contextual implementation factors relevant to low- and middle-income countries, with a particular focus on Libya. The most significant barriers identified were the absence of advanced molecular-biology infrastructure, limited bioinformatics expertise, and underdeveloped ethical and regulatory frameworks for genetic engineering research. At the same time, several facilitators emerged for regional shared-facility models, growing interest in bioinformatics training for pharmacy and medical students, and the potential for international collaborative partnerships. These findings suggest that a phased adoption strategy beginning with CRISPR-based target validation, progressing to organoid-based screening, and eventually expanding toward immune-cell engineering would maximize scientific productivity and educational benefits in Libyan pharmacology departments.

Table 4: Contextual barriers and facilitators for adoption

Domain	Barriers	Facilitators
Infrastructure	Limited advanced labs, lack of organoid culture platforms	Potential for shared regional facilities
Human capacity	Shortage of bioinformatics expertise	Strong pharmacy/medical student base for training
Regulation	Gaps in genetic engineering ethics and oversight	Opportunity for developing national regulatory frameworks
Funding	High upfront cost for CRISPR/immune-cell systems	International collaboration grants, phased adoption

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Discussion

This systematic review and meta-analysis evaluated the comparative performance of major genetic engineering tools CRISPR/Cas systems, RNAi, synthetic-biology organoids, and engineered immune-cell models in anticancer drug discovery. The findings confirm that these platforms have significantly advanced target validation, phenotypic screening fidelity, and translational relevance, while also highlighting persistent barriers to adoption in low- and middle-income settings. Consistent with earlier reports, CRISPR-Cas systems demonstrated superior accuracy and reproducibility compared with RNAi in functional genomic applications [5, 6]. The ability of CRISPR to introduce targeted double-stranded DNA breaks, yielding stable gene disruption, provides a robust and scalable foundation for drug target discovery [7]. Although RNAi remains less reliable due to incomplete knockdown and off-target silencing, its relative affordability and technical simplicity preserve its value as an entry-level platform, especially in resource-constrained laboratories [9, 10]. Synthetic-biology-based platforms, particularly organoid and three-dimensional culture models, were strongly associated with enhanced phenotypic relevance and reduced false-positive hit rates compared with conventional two-dimensional systems. These findings echo prior evidence demonstrating that organoids recapitulate tumor heterogeneity, genetic instability, and microenvironmental complexity more effectively, thereby improving predictive validity for therapeutic response [11-14]. For Libya and other LMICs, regional consortia or shared-infrastructure hubs may represent a pragmatic strategy to facilitate access to organoid technologies while distributing financial and logistical burdens [13].

Engineered immune-cell models, including CAR-T platforms, emerged as the most translationally impactful tools, consistent with recent breakthroughs in hematological malignancies [15-17]. However, their successful implementation demands sophisticated laboratory infrastructure, compliance with biosafety regulations, and rigorous ethical oversight. These challenges remain particularly acute in LMICs, where regulatory systems for advanced therapies are underdeveloped [17]. Nevertheless, phased introduction-via postgraduate research collaborations, pilot-scale studies, and international partnerships-may represent a feasible pathway for gradual adoption. The moderate heterogeneity observed across included studies ($I^2 \approx 40$-50%) is consistent with variations in experimental design, sample size, and institutional infrastructure. Importantly, the absence of major publication bias supports the robustness of pooled estimates, although underrepresentation of data from LMIC laboratories remains a concern. Similar asymmetry has been observed in other areas of biomedical research, where data from low-resource contexts are systematically less visible [20, 21]. Addressing this imbalance will require structural reforms such as open-access data platforms, regional research networks, and collaborative funding models [20, 21]. From a capacity-building perspective, the barriers identified in this review, limited molecular-biology infrastructure, insufficient bioinformatics expertise, and regulatory gaps-are significant yet not insurmountable. International experience demonstrates that training programs in computational genomics, cloud-based bioinformatics resources, and North-South research partnerships can substantially accelerate technology transfer [18, 19]. For Libya, a phased strategy is recommended: prioritizing CRISPR-based target validation, then integrating organoid platforms via regional collaborations, and eventually progressing toward immune-cell engineering as regulatory frameworks mature. This roadmap aligns ambition with feasibility, ensuring that the local pharmacology department advances in step with global innovation trajectories while maximizing both educational and translational impact. For pharmacology departments in Libya and similar low- and middle-income contexts, the adoption of these technologies will require a phased, resource-conscious strategy. Prioritizing CRISPR-based functional genomics, fostering shared regional facilities for organoid research, and integrating bioinformatics training into academic curricula represent realistic first steps. Long-term progress will depend on strengthening molecular biology infrastructure, addressing regulatory and ethical frameworks, and building international collaborations to ensure equitable participation in global innovation. Overall, the evidence underscores the transformative role

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of genetic engineering in reshaping anticancer drug discovery. Tailored implementation in resource-limited academic environments offers not only opportunities for scientific advancement but also a pathway for training the next generation of pharmacologists and researchers in alignment with global standards of precision medicine.

Conclusion: This study demonstrates that advanced genetic engineering tools particularly, CRISPR-Cas systems, RNAi platforms, organoid-based synthetic-biology models, and engineered immune-cell approaches, have substantially improved the precision, reproducibility, and translational relevance of anticancer drug discovery. CRISPR-based strategies emerged as the most effective for gene-target validation, organoids provided superior phenotypic modeling, and immune-cell engineering showed the greatest clinical promise, albeit with considerable infrastructural demands.

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